Complement

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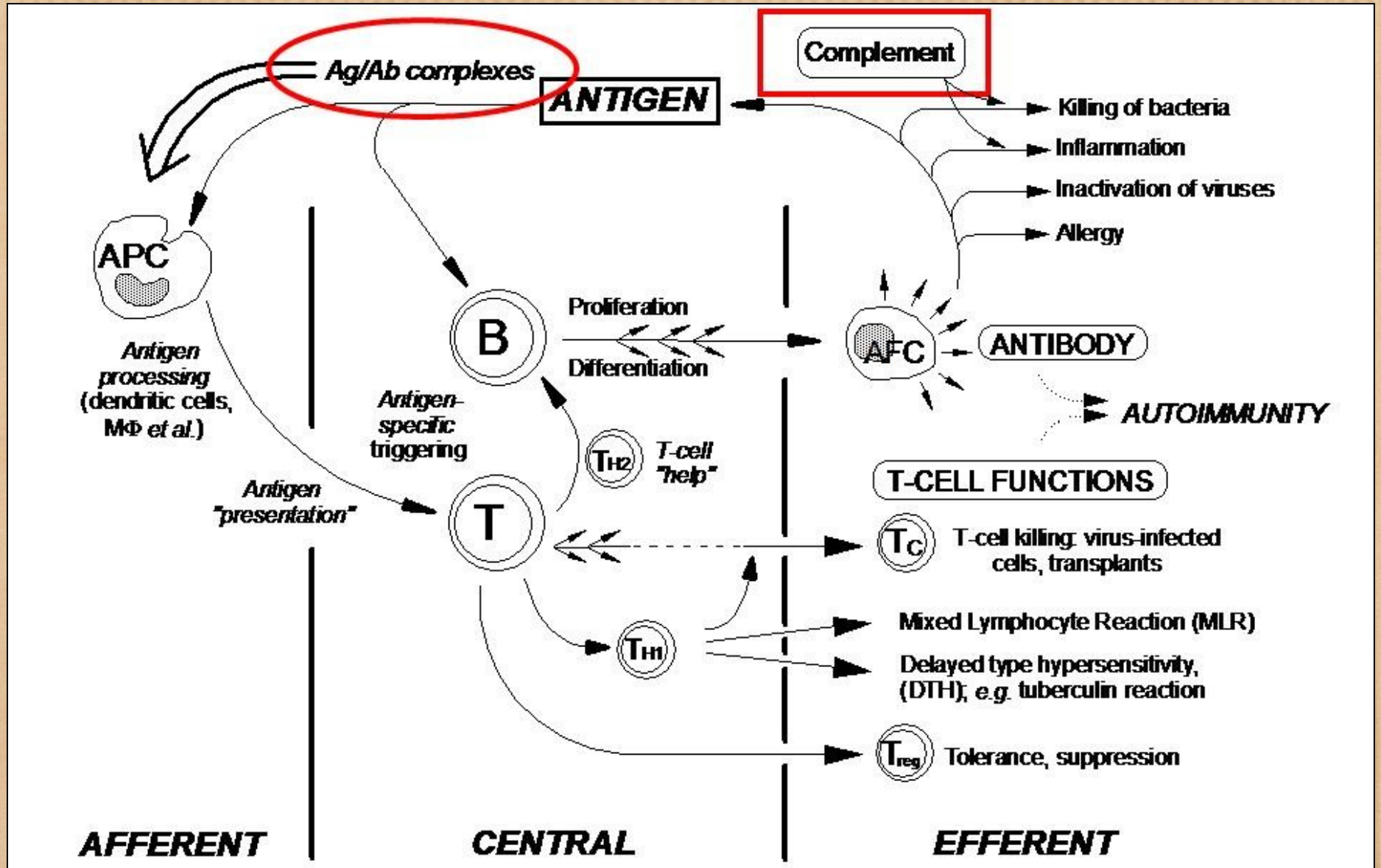
Micro / molecular Biology



THREE "LIMBS" OF THE IMMUNE RESPONSE

COMPLEMENT

Three Pathways:

CLASSICAL/ALTERNATE/MBLectin

INFLAMMATION

IMMUNE COMPLEXES

I.C. DISEASE

AUTOIMMUNITY

COMPLEMENT FIXATION ASSAY

Cardinal Signs of Inflammation

CALOR ***Heat*** *[↑Blood flow, C3a, C5a]*

RUBOR ***Redness*** *[↑Blood flow, C3a, C5a]*

TUMOR ***Swelling*** *[Accumulation of fluid & cells, C3a, C5a, C5b67]*

DOLOR ***Pain*** *[Swelling and tissue damage]*

…+ ***Loss of function***

May be initiated by…

Infection (bacteria, viruses et al.)

Sunburn, allergens…

Trauma…

Mediated by immune & coagulation systems, etc.

1894 – Pfeiffer, cholera vibrios are lysed in the peritoneum immune guinea pigs.

*1896 – **Bordet**, lysis of bacteria by immune sera requires two separate components...*

Lysis of Vibrio cholera by immune serum

Fresh normal serum	*no lysis*
Fresh immune serum	*lysis*
Heated immune serum	*no lysis*
Heated immune serum + fresh normal serum	*LYSIS*

Normal serum "complements" the ability of antibodies in immune serum to lyse targets

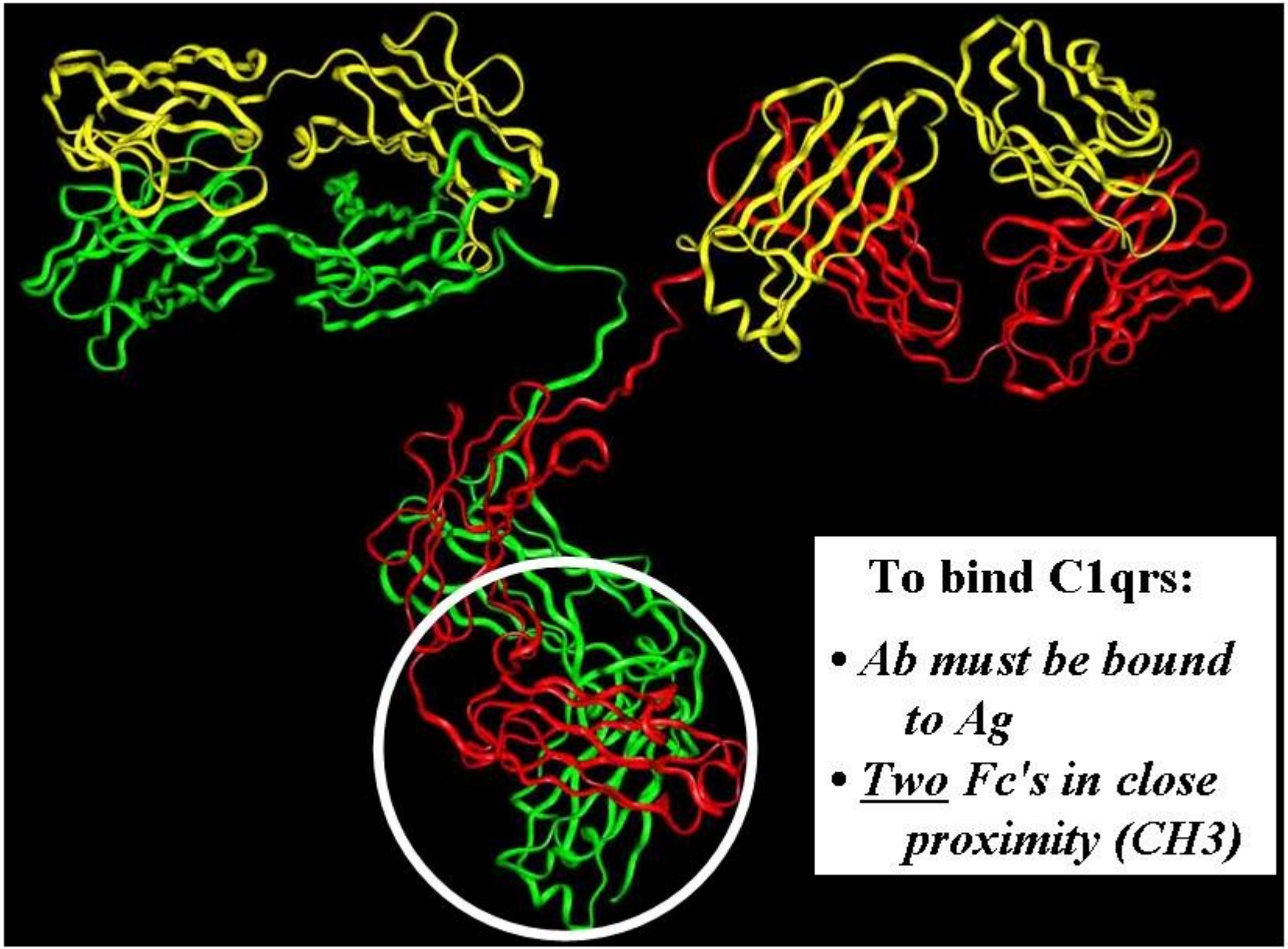
How is complement-mediated damage targeted?

***Antibodies*:**
Classical pathway (mostly)

***Unique structures on microbes*:**
Alternate & MBLectin Pathways

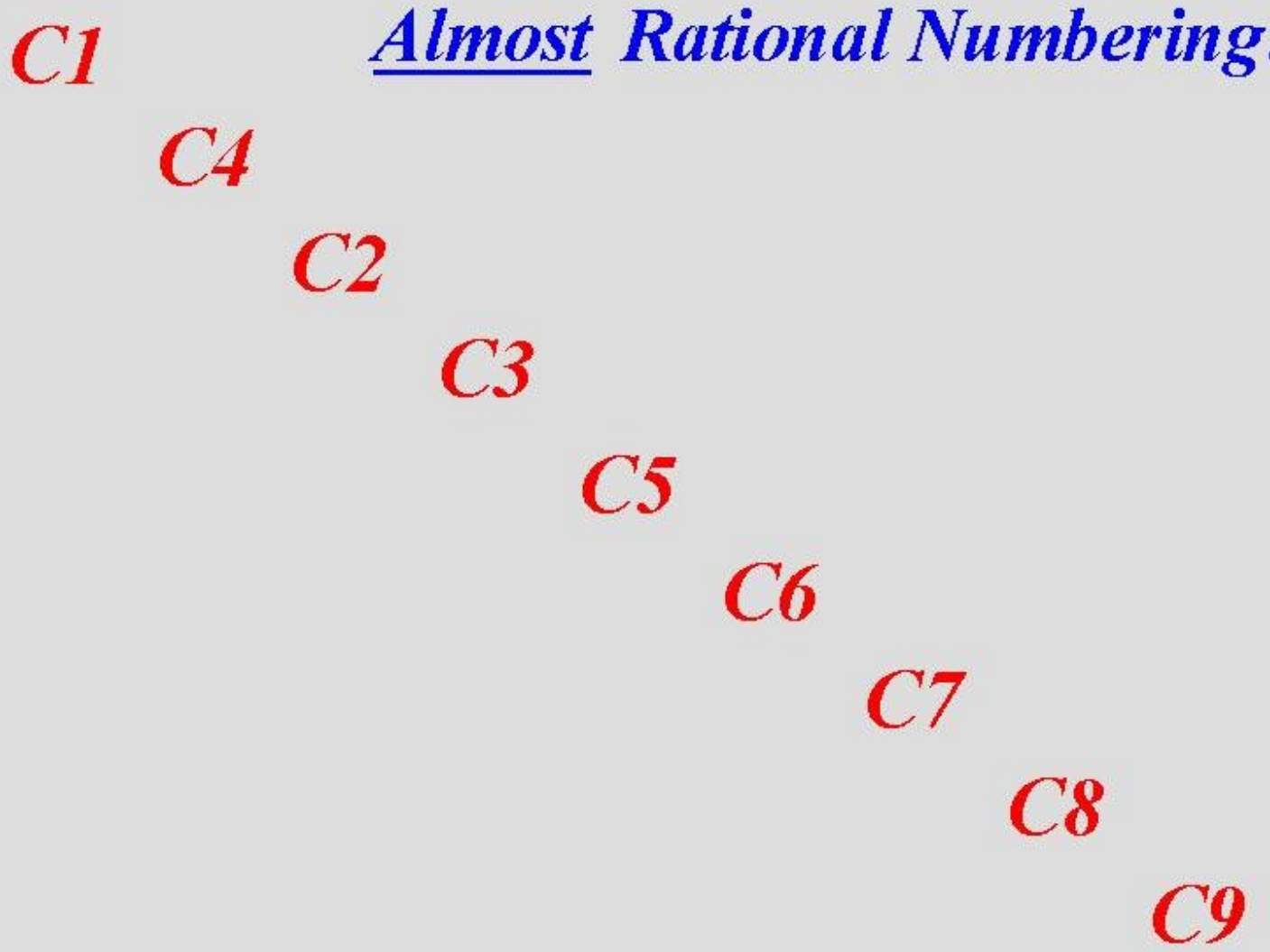
1) Classical Pathway

- *Initiated by AgAb complexes*
- *Protein/protein interactions*
- *Proteolytic cleavages and conformational changes…*
- *…which produce new enzymatic and biological activities*



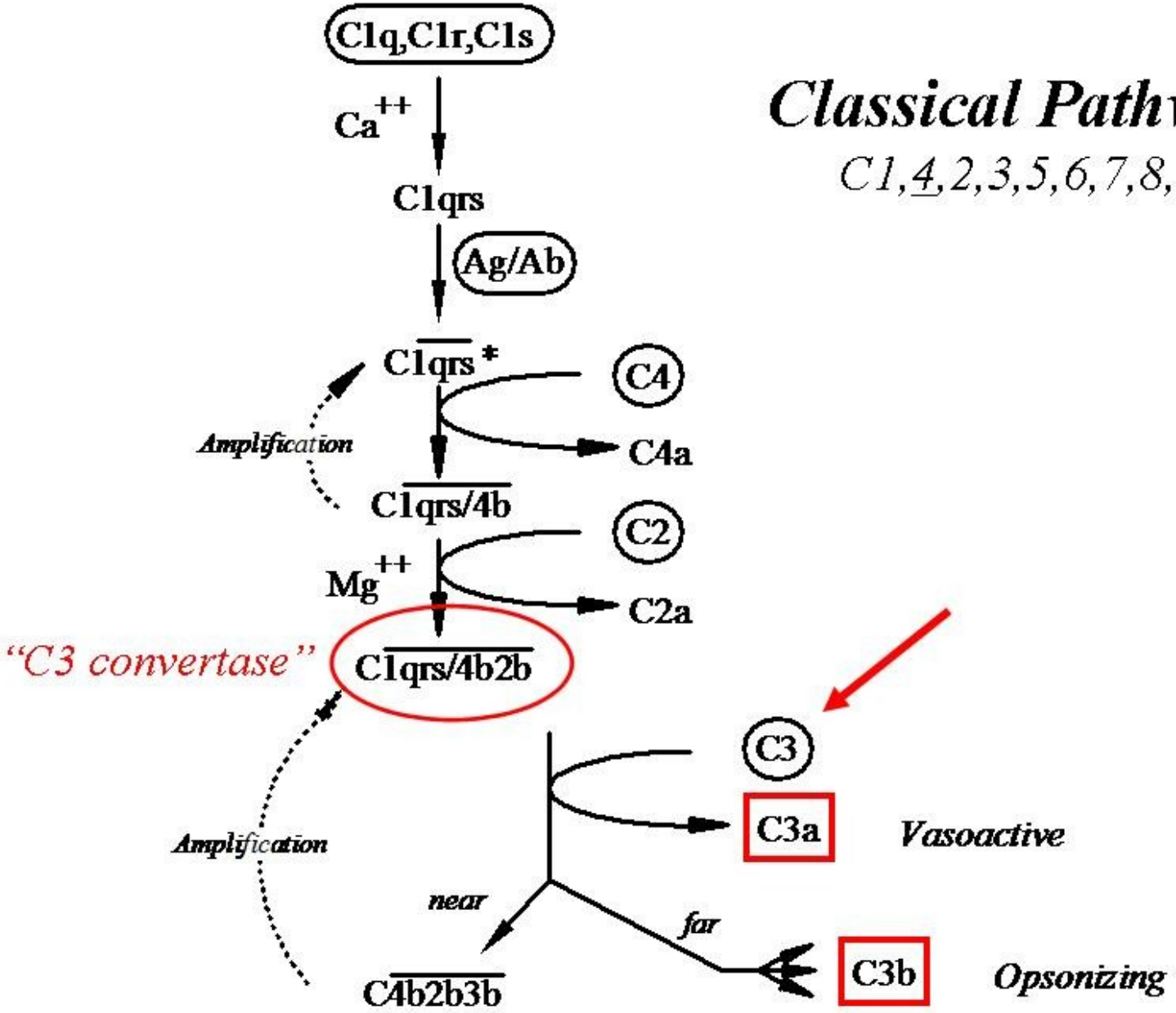
Structure of Mouse IgG2

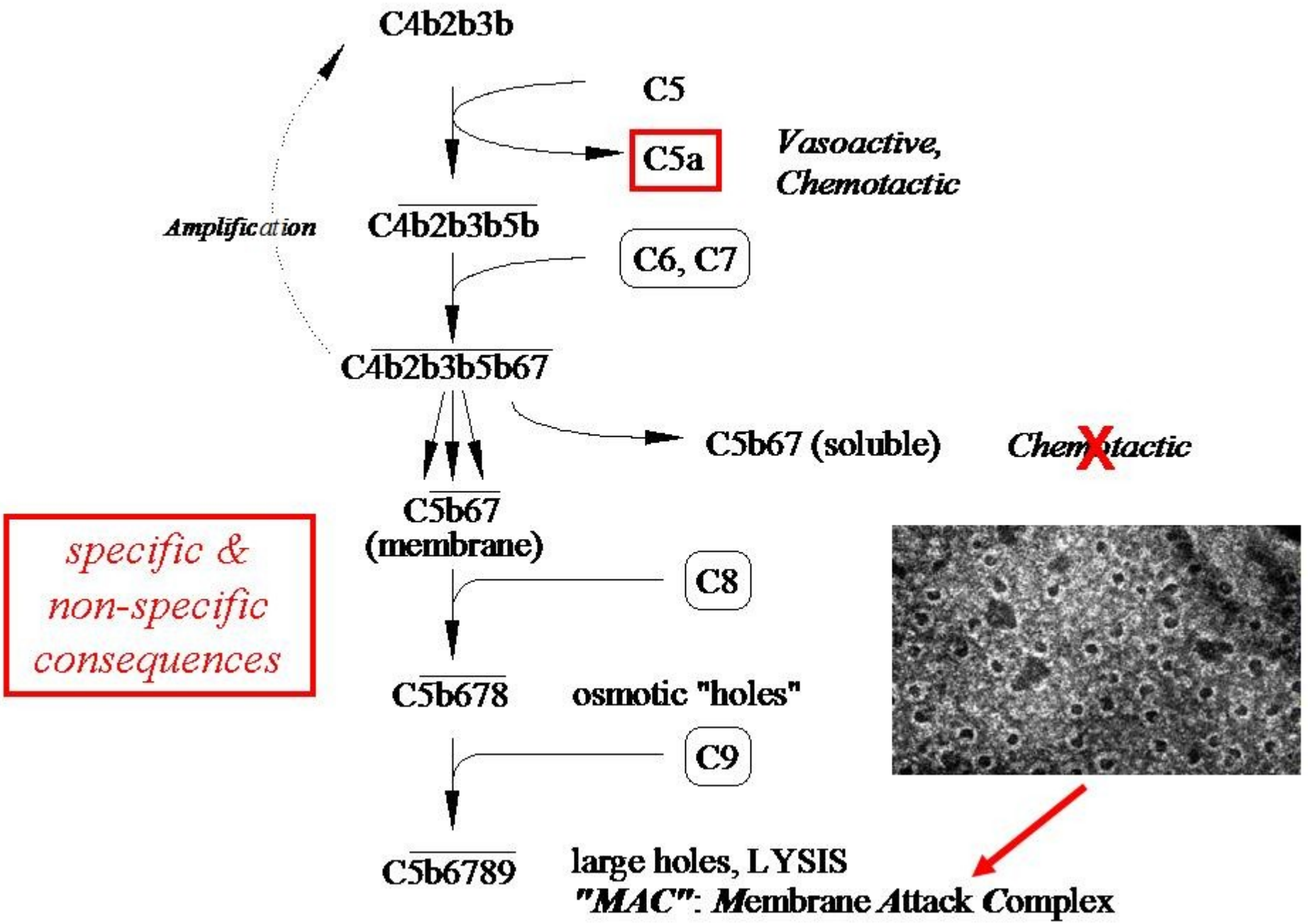
Harris et al., Nature 360:369-72, 1992


Almost Rational Numbering…
C1
C4
C2
C3
C5
C6
C7
C8
C9

Classical Pathway

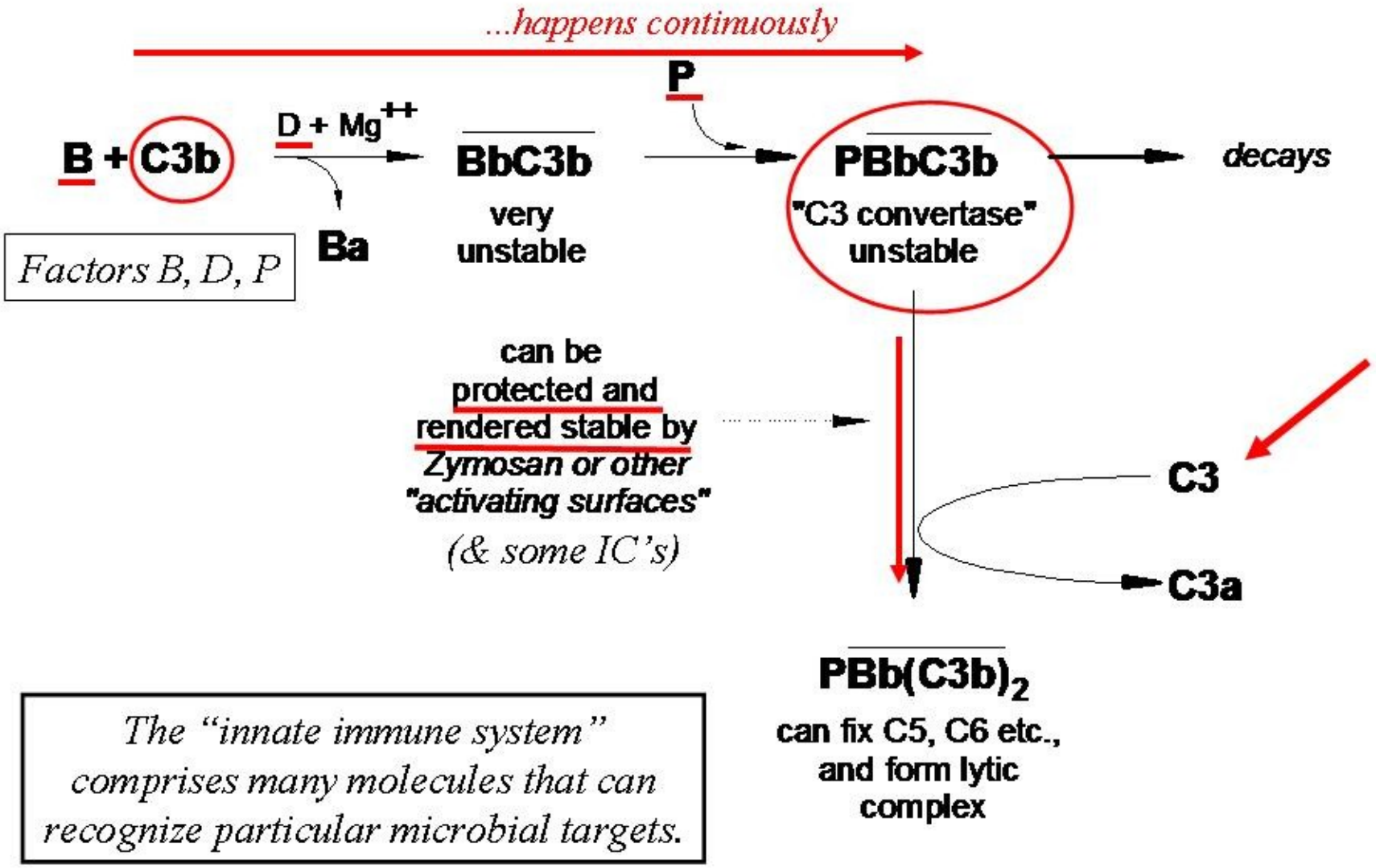
C1,4,2,3,5,6,7,8,9




C4b2b3b
C5
C5a
Vasoactive,
Chemotactic
Amplification
C4b2b3b5b
C6, C7
C4b2b3b5b67
C5b67 (soluble)
Chemotactic
C5b67
(membrane)
C8
C5b678
osmotic "holes"
C9
C5b6789
large holes, LYSIS
"MAC": Membrane Attack Complex
specific &
non-specific
consequences

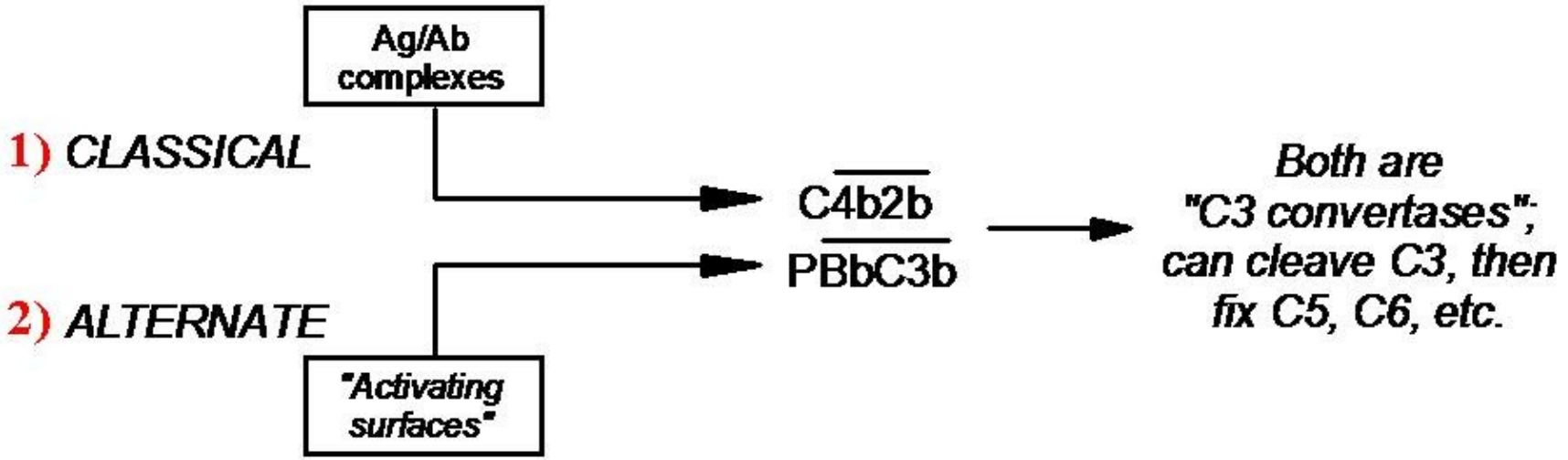
2) Alternate Pathway

- Initiated by microbial cell walls *et al*...



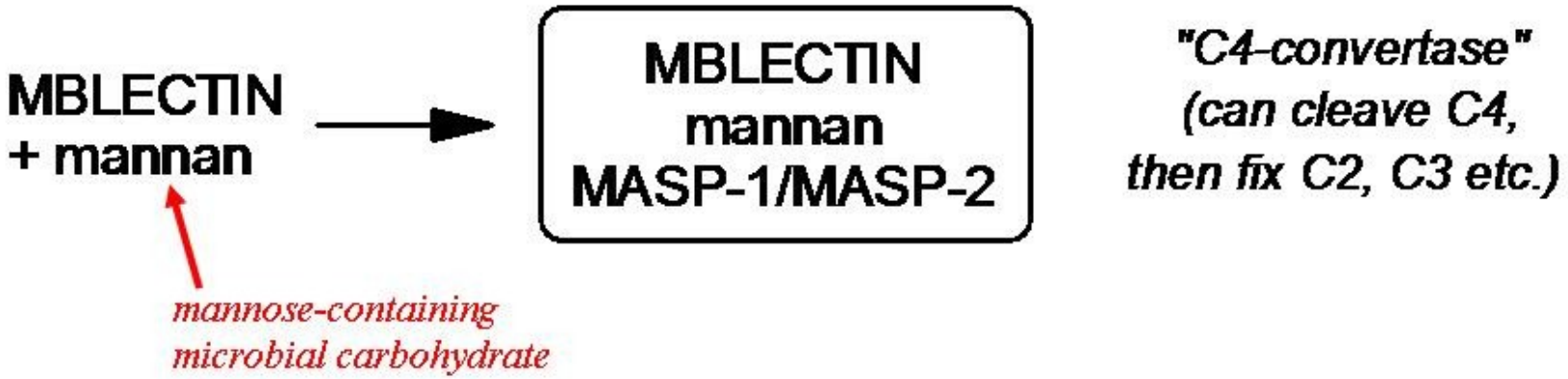
The "innate immune system" comprises many molecules that can recognize particular microbial targets.

RELATIONSHIP BETWEEN CLASSICAL AND ALTERNATE PATHWAYS



3) MBLectin Pathway

- Initiated by microbial carbohydrates…



Importance of antibody-independent complement fixation (Alternate & MBLectin pathways), speed of action:

What do you do before the fire engine arrives?

The Immune system as a defence organization

1. Its function is selective destruction.
2. It is large, complicated and elaborate.
3. It is expensive.
4. It is wasteful.
5. It has distinct components performing apparently identical functions.
6. It is slow to react.
7. It is prepared for events that never happen.
8. It fights today's threats with the solutions of past problems.
9. It is susceptible to corruption.
10. It can destroy that which it protects.

Peter Parham, 1990

Biological activities of Complement

1) ***Cytolysis***; disruption of cell membrane ("hemolysis").

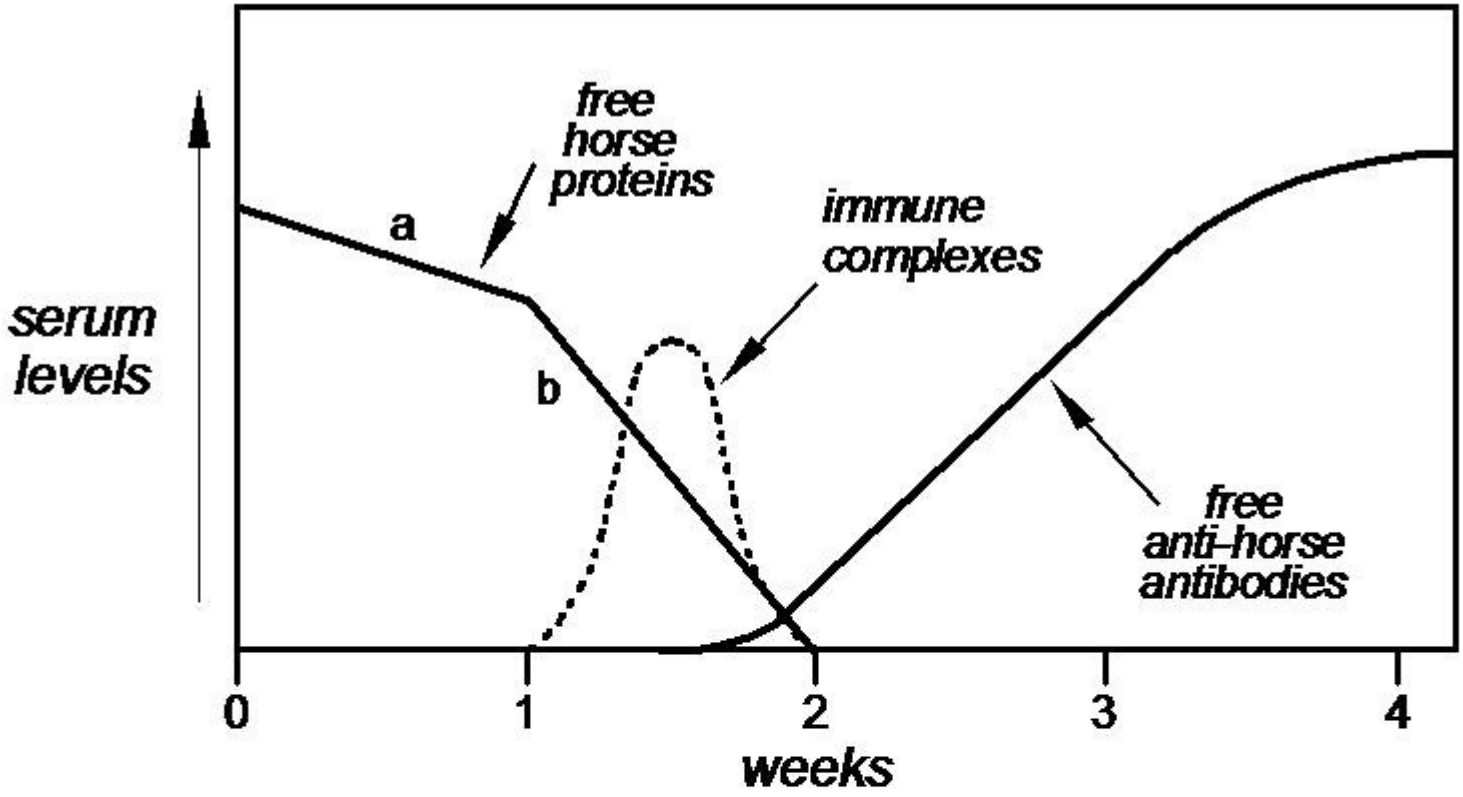
2) ***Anaphylotoxin activity***; ("vasoactive", "phlogistic"), increased capillary permeability, leakage of fluid.

3) ***Chemotaxis***; attraction of PMN's.

4) ***Opsonization***; phagocytosis by macrophages & PMN's.

5) ***Tissue damage***; effects of lytic complex and accumulation of PMN's (Arthus reaction, Immune Complex Disease).

Inflammation: heat, redness, swelling, pain, loss of function...



ONE-SHOT SERUM SICKNESS

Initiated by a single dose of horse anti-diptheria toxin

Transient Immune Complex Disease

Passive immunization

Remember: Active immunization preferable to passive...

Human anti-toxins preferable to animal...

Different Routes to Immune Complex Reactions

Intravenous: Drugs, anti-toxins & anti-venins, therapeutic mAbs

Subcutaneous: Vaccination (tetanus)

(classic Arthus skin reaction…)

Inhaled: "Farmer's Lung" (actinomycetes *et al.*)

May also be autoimmune (e.g. SLE, RA)

COMPLEMENT DEFICIENCIES

C5-9 (MAC): *Limited susceptibility* to infections (!)

C3, P, B: *More severe* infections

MBL: Mainly childhood infections

Supportive therapy; treat infections, fresh-frozen plasma to replenish complement levels.

***C1,4**: Increased risk of developing autoimmune disease*

Active in soluble phase, important in normal clearance of trace amounts of immune complexes.

C4b receptors (CR1) on RBC transport ICs to liver where they are removed.

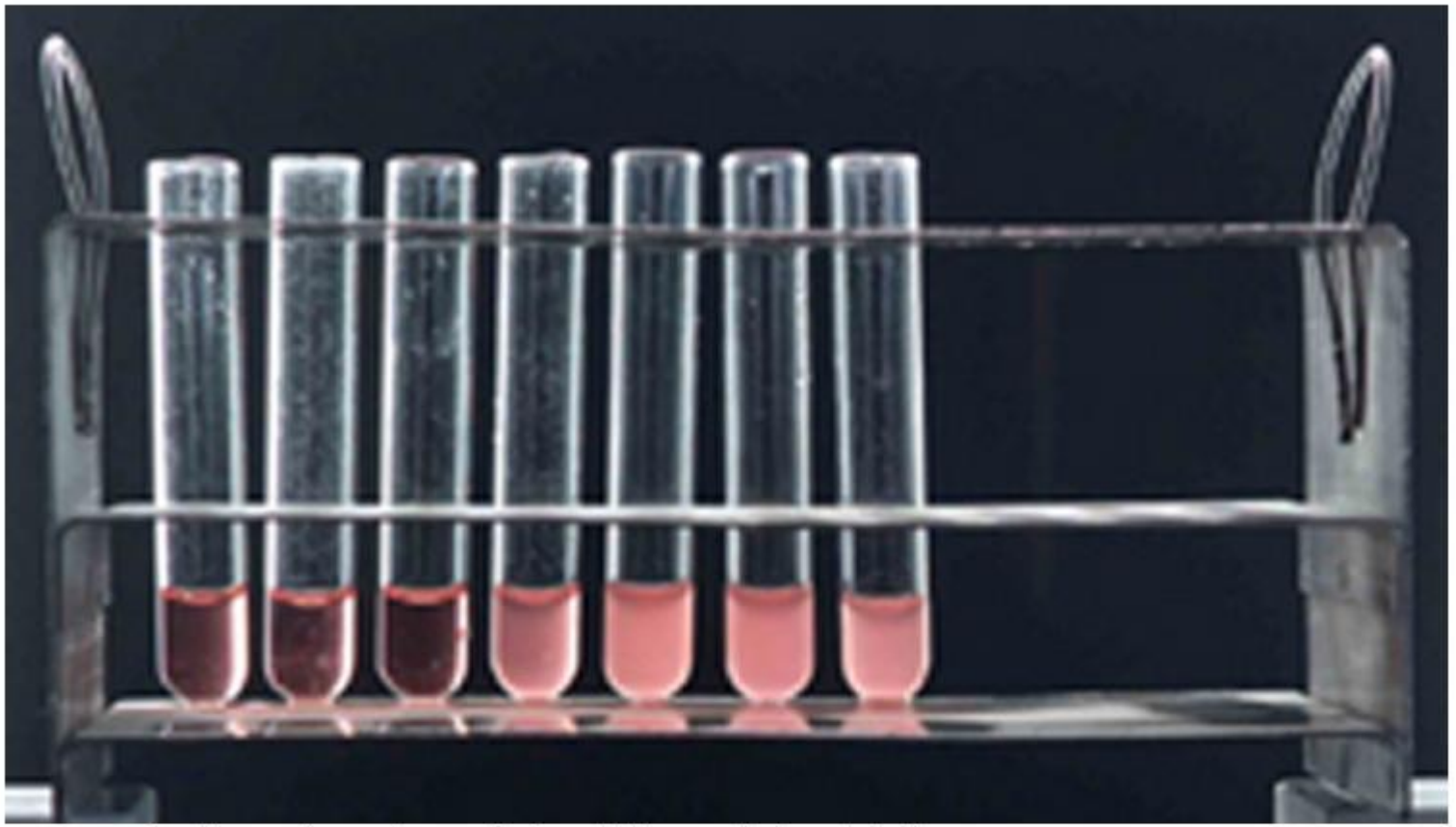
Measuring Antibody/Antigen Reactions

1) Precipitation: ***Ouchterlony***

2) Solid-state binding, labelled antibody: ***RIA/ELISA***

3) Liquid-phase binding: ***Equilibrium Dialysis***

4) Binding of complement: ***Complement Fixation***
(hemolysis)



1:2 4 8 16 32 64 128

fresh guinea pig serum

Titration of complement on sensitized RBCs ("EA")

Basis for Complement Fixation Assay

Ab + C + EA Lysis

Ab + *Ag* + C

→ Ab/*Ag* + C

→ Ab/*Ag*/C + EA *No Lysis*

Presence of antigen is made visible by inhibition of lysis.